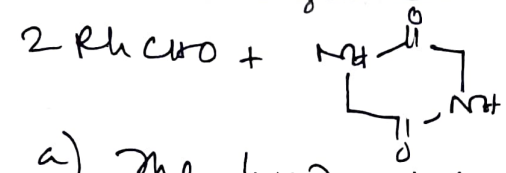
Biomolecules

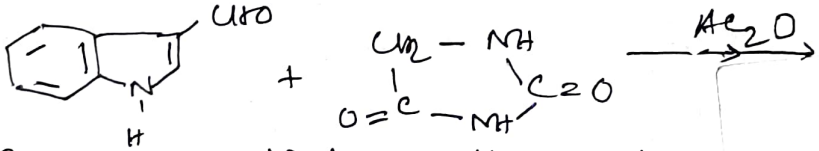
Q) a) Discuss the hydantoin synthesis of α-amino acids. Give an Example.

b) A modification of the hydantoin synthesis is the Bucherer hydantoin synthesis. Discuss this method and give an Example.

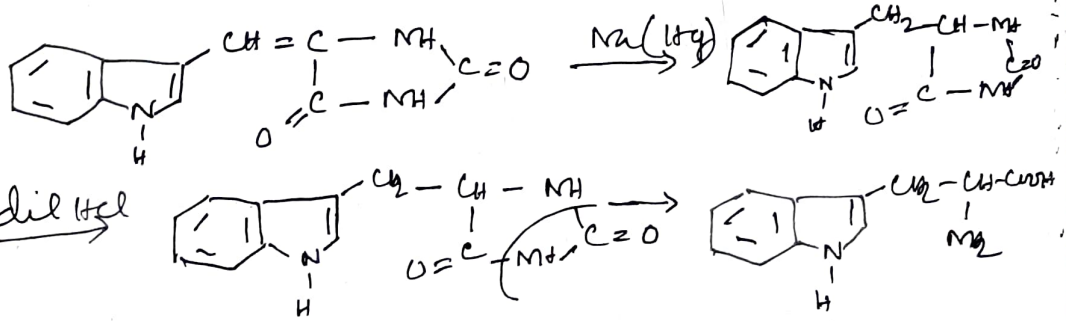
c) Identify A and B in the following reaction sequence:



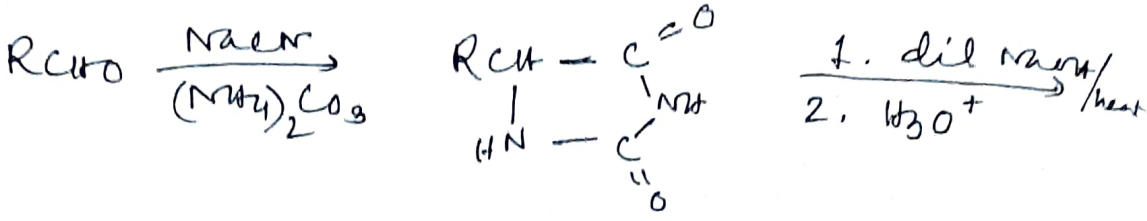
Ans: a) The hydantoin synthesis involves condensation of an aromatic aldehyde with hydantoin. The product so obtained is reduced with sodium amalgam or ammonium hydrogen sulphide followed by hydrolysis, to yield an α-amino acid. Tryptophan, for example, can be synthesised from Indole-3-aldehyde (Prepared from indole by means of the Reimer-Tiemann reaction) by this method.



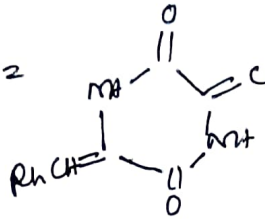
Indole-3-aldehyde Hydantoin



b) In Bucherer hydantoin synthesis an aldehyde is first converted into a 5-substituted hydantoin by means of ammonium carbonate and sodium cyanide in aqueous ethanol solution. The resulting substituted hydantoin upon alkaline hydrolysis followed by acidification gives an α-amino acid

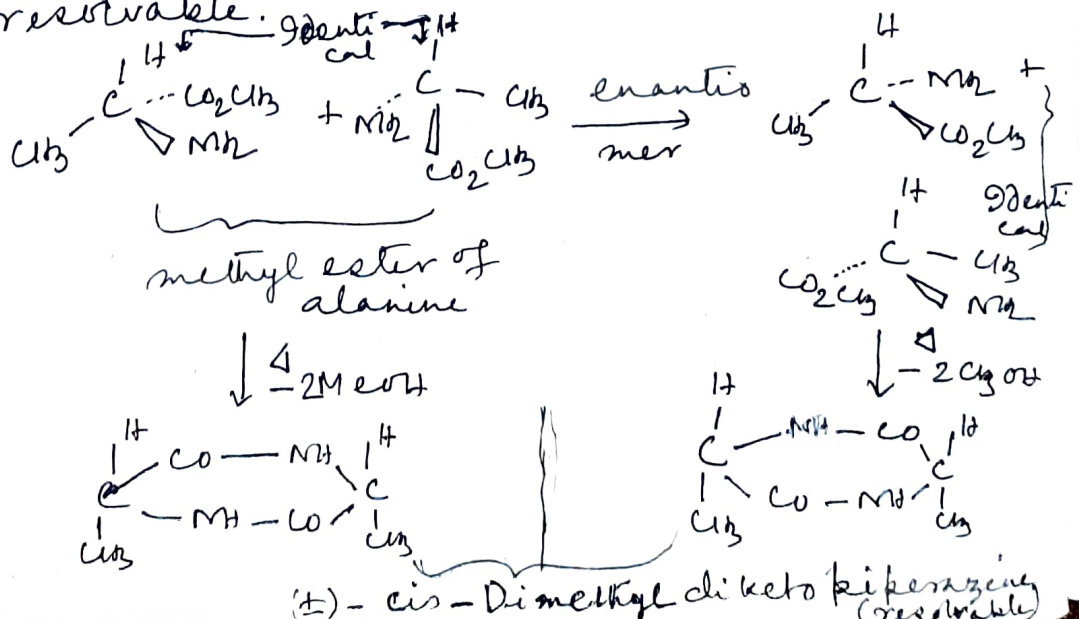


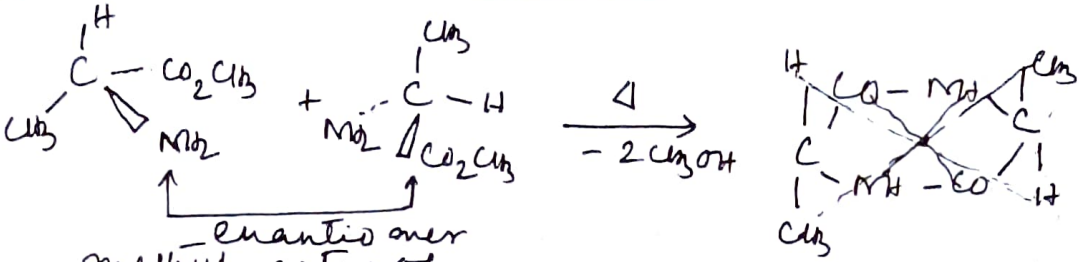
$R-CH(\overset{+}{N}H_3)-COO^-$ An α-amino acid.

c) A =  ; B = $PhCH_2CH(NH_2)COOH$

Q. When the methyl ester of (±) - alanine is heated, two diastereoisomeric dimethyl diketo piperazines are obtained. One of them cannot be resolved. Give their structures and account for their stereo chemistry.

Ans:- When heated, methyl ester of (±) - alanine (two molecules) reacts intermolecularly to give cis and trans - dimethyl diketo piperazine (two geometric isomers i.e. diastereo isomers). The cis isomer is chiral and therefore resolvable where as the trans isomer with a centre of symmetry is achiral (a meso - compound) and not resolvable.





enantiomer methyl ester of alanine

Trans - Dimethyl diketo piperazine. (a - meso compound)

Q. Explain and define isoelectric point of amino acids. Show that isoelectric point (PI) of a α-amino acid like $NH_2-\underset{R}{CH}-COOH$ is given by $PI = \frac{pK_{a_1} + pK_{a_2}}{2}$ where K_{a_1} and K_{a_2} are first and second dissociation constants of dipolar form of a α-amino acid.

Ans: We find that in case of glycine, acidic condition stabilises $\overset{+}{N}H_3CH_2COOH$ and basic condition, $NH_2CH_2COO^-$. Therefore, ionic states of glycine depend on P_H of the medium. In acidic P_H, it remains as cation and in basic medium as anion. The position of the equilibrium depends on the P_H of the solution. For each amino acid there exists a particular P_H value at which the concentration of dipolar ion is maximum. Under this condition, the dipolar ion is said to be electrically neutral (net charge is zero) and the said amino acid does not migrate in any direction when placed in the electric field. This particular P_H - value at which an amino acid behaves as an electrically neutral species is known as the isoelectric point of that amino acid.

Isoelectric point of a neutral amino acid is found to half of the two pK_a values; one obtain

- ed as a conjugate acid and other as a dipolar ion. This has been shown below taking glycine as an example.

In case of glycine, the first dissociation refers to ionisation of $-COOH$ group of $\overset{(+)}{N}H_3CH_2COOH$ (conjugate acid) and the second dissociation is the ionisation of H^+ from $-\overset{(+)}{N}H_3$ part of $\overset{(+)}{N}H_3CH_2COO^-$ (dipolar ion) on the basis of this fact, the necessary calculations can be shown as follows.

1st dissociation:

$$\underset{\text{Conjugate acid of glycine}}{\overset{+}{N}H_3CH_2COOH} \overset{K_{a_1}}{\rightleftharpoons} \underset{\text{Dipolar ion}}{\overset{(+)}{N}H_3CH_2COO^-} + H^+$$

$$K_{a_1} = \frac{[\overset{+}{N}H_3CH_2COO^-][H^+]}{[\overset{+}{N}H_3CH_2COOH]} \quad \text{or} \quad [\overset{+}{N}H_3CH_2COOH] =$$

$$\rightarrow \frac{[\overset{+}{N}H_3CH_2COO^-][H^+]}{K_{a_1}}$$

2nd dissociation:

$$\underset{\text{Dipolar ion}}{\overset{+}{N}H_3CH_2COO^-} \overset{K_{a_2}}{\rightleftharpoons} NH_2CH_2COO^- + H^+$$

$$K_{a_2} = \frac{[NH_2CH_2COO^-][H^+]}{[\overset{+}{N}H_3CH_2COO^-]} \quad \text{or,} \quad [NH_2CH_2COO^-] = \Rightarrow$$

$$\frac{K_{a_2}[\overset{+}{N}H_3CH_2COO^-]}{[H^+]}$$

At the iso electric point (P_H) the concentration of the dipolar ion is maximum and since in that condition the system is electrically neutral, we can conclude, that ~~was~~ at the iso electric point the concentration of conjugate acid is equal to that of conjugate base.

$$\frac{[\overset{+}{N}H_3CH_2COO^-][H^+]}{K_{a_1}} = \frac{K_{a_2}[\overset{+}{N}H_3CH_2COO^-]}{[H^+]}$$

Therefore, $[H^+][H^+] = K_{a_1} \times K_{a_2}$

or, $[H^+]^2 = K_{a_1} \times K_{a_2}$

or, $2pH = pK_{a_1} + pK_{a_2}$

or, $pH = \frac{pK_{a_1} + pK_{a_2}}{2}$

Therefore iso electric point $pI = \frac{pK_{a_1} + pK_{a_2}}{2}$

$pI = \frac{pK_{a_1} + pK_{a_2}}{2}$

In case of glycine $pK_{a_1} = 2.4$ and $pK_{a_2} = 9.78$

Therefore, iso electric point,

$$pI = \frac{2.4 + 9.78}{2} = 6.07.$$

Q: What is called Zwitter ion or inner salt?

Ans:- Addition of an amine to a carboxylic acid gives ammonium salt of the amine group. This occurs by the transfer of a proton from the carboxylic group to the amino group. However, when both these groups form part of the same molecule, as indeed is the situation in amino acids, the proton transfer occurs intramolecularly to give what is called a dipolar ion or zwitter ion or inner salt, a form in which the carboxylic group is present as a carboxylate ion, $-CO_2^{(-)}$, and the amino group is present as an ammonium group, $-NH_3^{(+)}$. Because amino acids contain both acidic ($-NH_3$) and basic ($-CO_2^-$) groups, they are amphoteric (having both acidic and basic properties) i.e. they can accept a proton from a stronger acid as well as donate a proton to a stronger base. In aqueous solutions the amino acids exist as the following species which are in equilibrium.

$$NH_3^+ - CH - COOH \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} NH_3^+ - CH - CO_2^-$$
$$\quad R \qquad\qquad\qquad\qquad\qquad\quad R$$

Cationic form (predominant in strongly acidic solutions, e.g. at $P_H = 0$)

Dipolar ion or zwitter ion

$NH_2-CH(R)-CO_2H$ (minor neutral form)

$NH_2-CH(R)-CO_2^{(-)}$

Anionic form (Predominant form in strongly basic solutions e.g. at P_H 12)

The predominant form of the amino acid present in a solution depends on the P_H of the solution and on the nature of the amino acid. In a strongly acidic solution, the $CO_2^{(-)}$ group is protonated to a free $-CO_2H$ group, and the molecule has an overall positive charge, i.e, the amino acid exists primarily as a cation. As the P_H is raised, the $-COOH$ group loses its proton and at about P_H 2 half of the cationic form will be converted to the dipolar ion. As the P_H is raised further, the $-NH_3^{(+)}$ group loses its proton also at about P_H 9-10 the dipolar ion will be half converted to the anionic form. Then as the P_H approaches P_H 12, the anionic form becomes the predominant form present in the solution.

9. a) What is called electrophoresis?
b) How are amino acids separated and identified by electrophoresis?
c) What is suitable P_H for separating a mixture of aspartic acid, histidine and threonine by electrophoresis? Give your reasoning.
d) How can lysine (PI = 9.7) be separated from alanine (PI = 6.0) by electrophoresis?

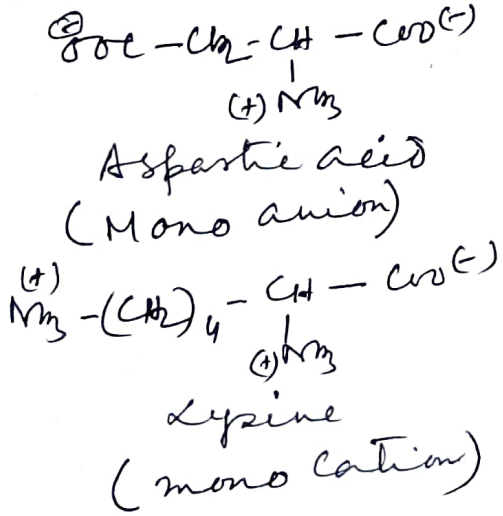
Ans: a) Electrophoresis is a method of separation and purification of amino acids that relies on the movement of charged particles in an electric field.

b) Differences in isoelectric points can be used to separate mixtures of amino acids by electrophoresis.

A streak of the amino acid mixture is placed in the centre of a layer of acrylamide gel or a piece of filter paper and the paper or the gel is placed in a buffer solution. Then, the two electrodes are placed in contact with the edges of the gel or paper and an electric field is applied. An amino acid with an overall positive charge migrates towards the negative electrode. An amino acid with an overall negative charge migrates toward the positive electrode. An amino acid with P_I (isoelectric point) equal to the P_H of the buffer solution has no net charge (because it exists as a dipolar ion) so it does not move. If two molecules have the same charge, the larger one will move more slowly during electrophoresis because the same charge has

to move a greater mass. Thus if a mixture containing alanine, lysine and aspartic acid is subjected to electrophoresis in a buffer that matches the iso electric point of alanine ($P_H = 6.0$), Iso electric point of alanine ($P_I = 6.0$), Aspartic acid ($P_I = 2.8$) which is in the anionic form (because P_H 6 is less acidic than its $P_I = 2.8$) migrates towards the positive electrode (the anode), alanine which has zero net charge remains at the origin, and the lysine ($P_I = 9.7$) which is in the cationic form (because $P_H = 6$) is more acidic than its PI) migrates towards the negative electrode (the cathode). After a period of time, the separated amino acids are received by cutting the paper or scraping the bands out of the gel.

Structure at P_H 6 (Buffer solution)



Aspartic acid (Mono anion)

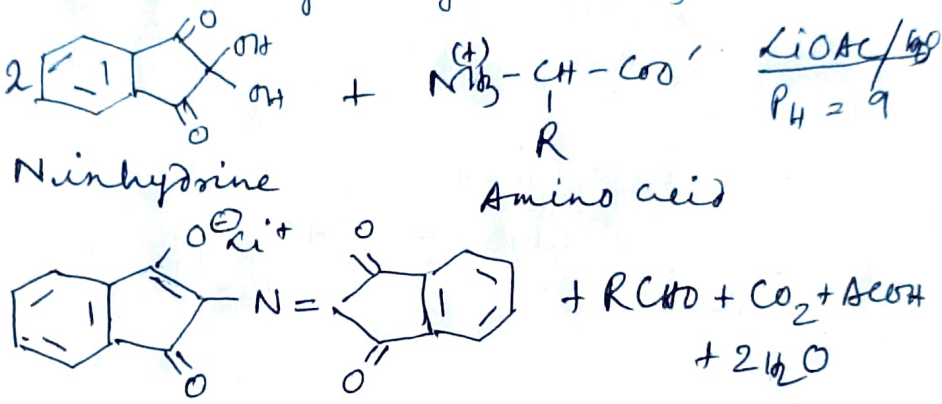
Alanine (neutral)

Lysine (mono cation)

Q. Discuss the reaction of amino acid with Ninhydrine. Discuss the mechanism.

Ans:- When Ninhydrine reacts with an aqueous solution of an amino acid in the presence of LiOAc ($P_H = 9$), an intensely coloured purple (called Ruhemann's purple)

is obtained. It is this purple colour that is used to detect amino acids. All amino-acids except proline and hydroxyproline, regardless of the identity of the R-groups, form the same purple molecules on reaction with Ninhydrine.
and this is because in this reaction the R-group of the amino acid is lost as an aldehyde
The reaction may be given as follows.



Ruhemann's Purple

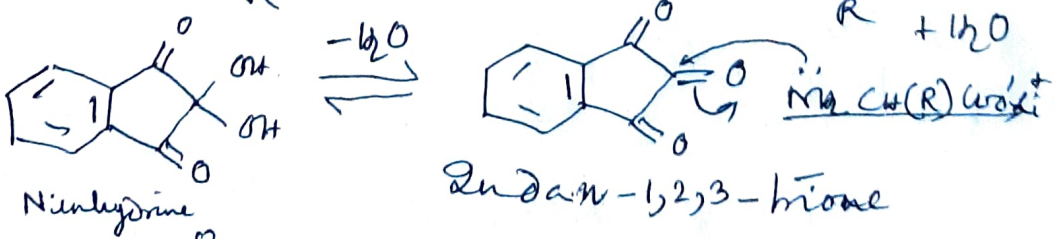
Mechanism:

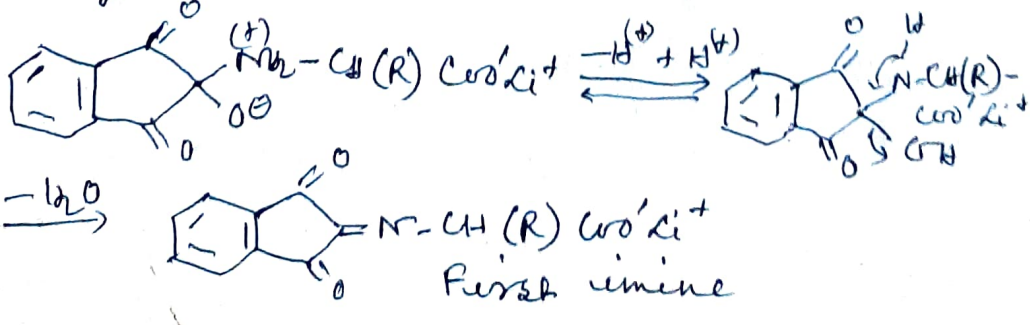
The reaction proceeds through the steps as follows:

Step 1: Formation of an imine (a Schiff's base)

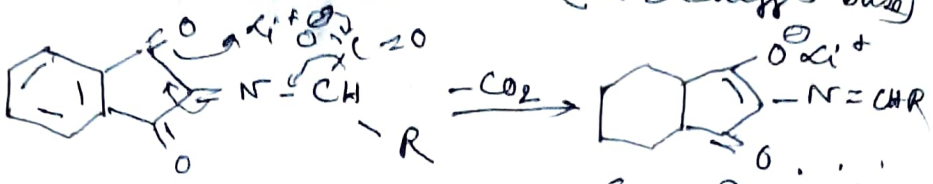
$$Li^+OAc^- + H_2O \rightleftharpoons (Li^+ + OH^-) + AcOH$$

$$NH_3^+ - CH(R) - COO^- + LiOH \rightleftharpoons NH_2 - CH(R) - COO^-Li^+ + H_2O$$



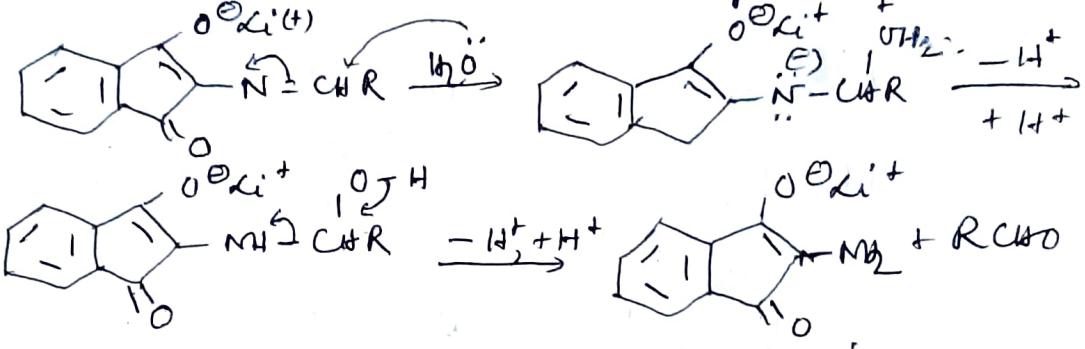


Step II : Decarboxylation of the resulting imine to give a second imine (a Schiff's base)



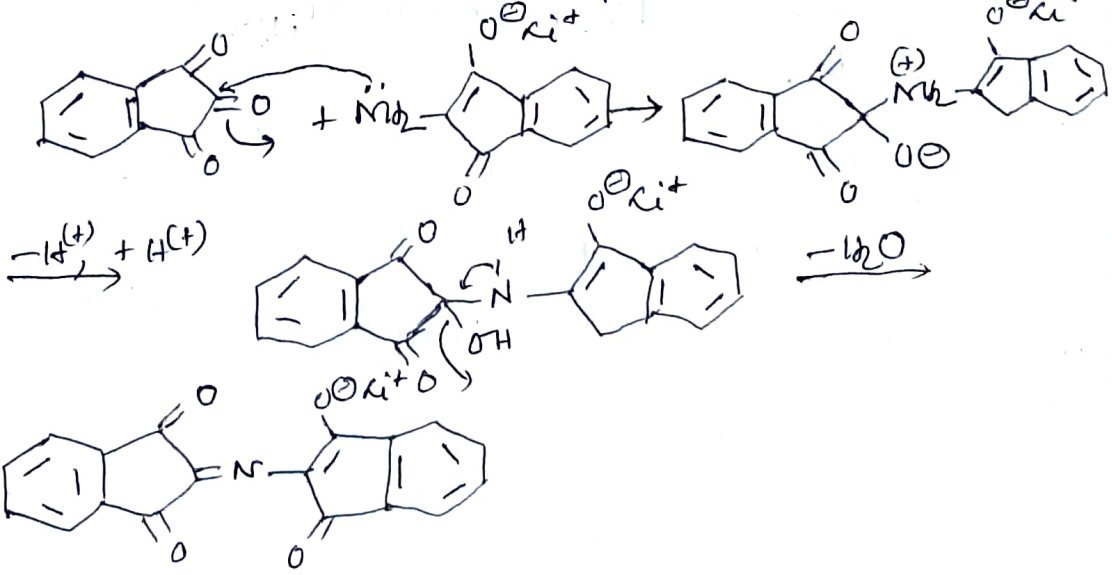
Second imine

Step III : Hydrolysis of the second imine to form an amine and an aldehyde.



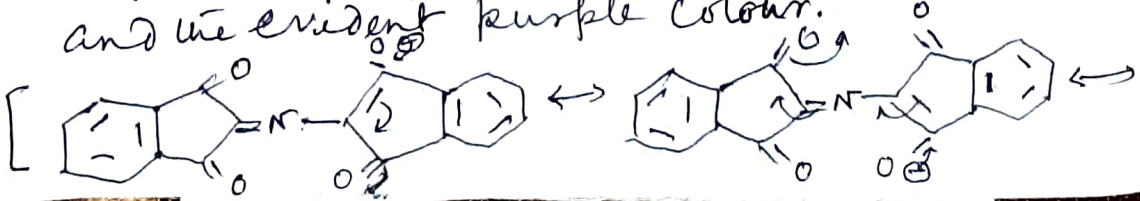
An amine

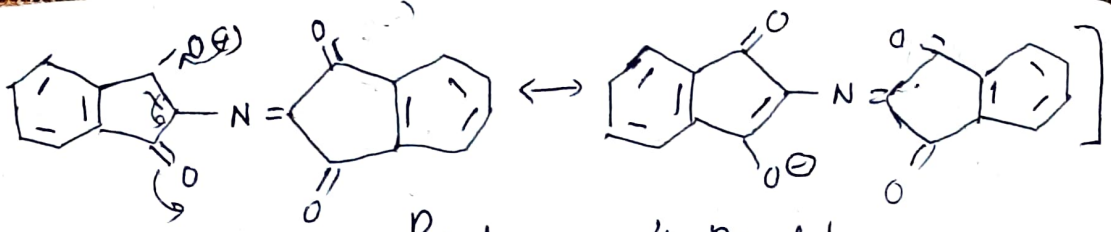
Step IV : Condensation of the amine with a second molecule of 2nd an-1,2,3-trione to form the purple coloured product.



Ruhemann's Purple

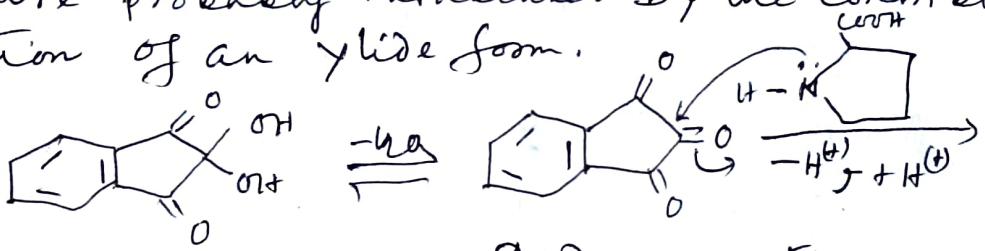
The extensive conjugation in the product leads to a very low energy absorption in the visible region and the evident purple colour.



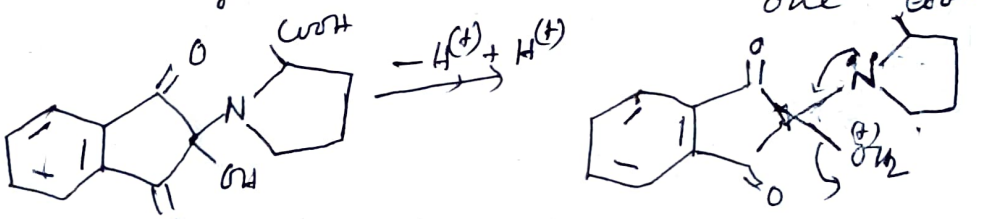


Ruhemann's Purple.

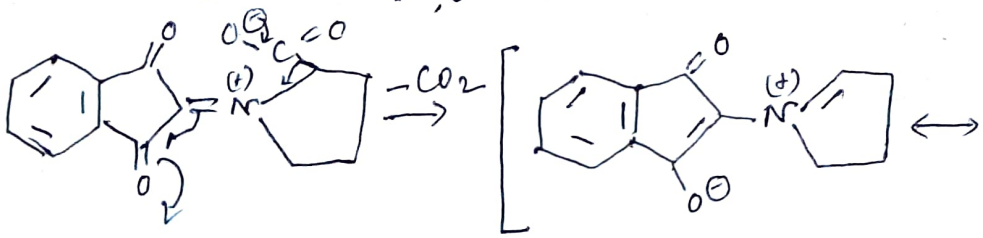
Proline and hydroxy proline donot react with Ninhydrine in the same way because their α-amino groups are part of a five membered ring. They give different compounds on reaction with Ninhydrine. These absorb light at different wavelengths, therefore, donot give purple colour rather a yellow colour. Proline reacts to give the following yellow compound with a structure probably stabilised by the contribution of an ylide form.

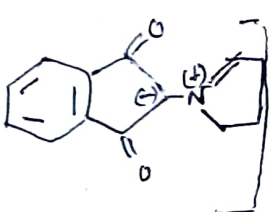


Ninhydrine



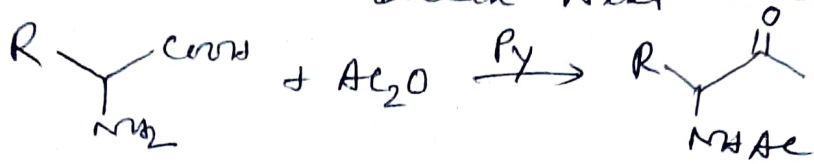
2nd an-1,2,3 tri one





DAKIN-WEST reaction :-

When an α-amino acid is treated with acetic anhydride in the presence of pyridine, the carboxyl group is replaced by an acyl group and the amino group becomes acylated to give α-acetamido ketone. This is called Dakin-West reaction.



α-Acetamido ketone

The mechanism is believed to go via oxazolone (azlactone) intermediate.